

DOCKET NO.: CACO-0067 (P21303US)
PATENT APPLICATION

INT'L. SERIAL NO.: PCT/GB00/00157
INT'L. APPL. FILED: JANUARY 21, 2000

At page 22 replace the references spanning lines 2 - 5 with the following replacement references:

- 38
1. **Knox, R. J.** (Personal communication).
 2. **Hendrix, R. W., J. W. Roberts, F. W. Stahl, and R. A. Weisberg.** (1983)
Lambda II, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
 3. **Murray, N. E.** (1983) Bacteriophage lambda and molecular cloning, p. 395-431.
In Hendrix *et al.*, (eds.) Lambda II, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

REMARKS

The specification has been amended to insert appropriate sequence identification numbers (SEQ ID NOs), to correct minor typographical errors, and to correct obvious omissions or errors in reference citations. No new matter has been added.

Conclusion

Applicant respectfully submits that claims 1 - 21 are in condition for allowance. Applicant respectfully requests early notification of the same. If a telephonic interview would

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be helpful, the Examiner is asked to call the undersigned at 215-557-5901. Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached pages are captioned "**Version with markings to show changes made.**"

Respectfully submitted,



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Date: *November 6, 2001*

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

Paragraph on page 12 spanning lines 11 - 14:

Suitable media in which to carry out the method of the present invention will be clear to those of skill in the art. For example, for *E. coli*, [Luria-Bertani] Luria-Bertani (LB) broth is suitable. In addition to prodrug, the media may contain other necessary additives, for example, antibiotic or an inducer, for example, IPTG.

Paragraph on page 13 spanning lines 18 - 24:

A DNA fragment containing the tac promoter was obtained from plasmid pDR540 (Pharmacia), by PCR amplification using primers 5'- ACCTGACGTCTAAGAAAC -3' (SEQ ID NO:1) and 5'- GCTCTAGATTGTTATCCGCTCAC -3' (SEQ ID NO:2). The amplified DNA fragment was cleaved with restriction endonucleases *EcoRI* and *XbaI*, and the major fragment (369 bp) was cloned between the *EcoRI* and *XbaI* sites of the widely used vector plasmid, pUC19, to generate plasmid pPS1133C2. The sequence of the insert was verified by DNA sequencing (Sanger [& Coulson], *et al.* 1977, Proc. Natl. Acad. Sci. USA, 74: 5463-7).

Paragraph on page 14 spanning lines 2 - 7:

The two oligonucleotides PS1133A (5'- CTAGGGCCTGCGAGGCCTTAATTAA-GGCCTCCCGGGCCT -3') (SEQ ID NO:3) and PS1133B (5'- CTAGAGGCCCGGGAGGCCTTAA[-]TTAAGGCCTCGCAGGCC -3') (SEQ ID NO:4) were annealed together to generate a short piece of DNA containing two *SfiI* sites separated by a *PacI* site, and with 4 nucleotide 5' extensions on either end compatible with ligation into *XbaI* sites. However, only that at the right end regenerates the *XbaI* site:

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Paragraph on page 14 spanning lines 17 - 20:

The plasmid pET11c (Novagen) was cleaved with *Xba*I, and the following annealed oligonucleotides were cloned into that site:

PS1134A 5' CTAGAGGCCTGCGAGGC 3' (SEQ ID NO:5)
PS1134B 3' TCCGGACGCTCCGGATC 5' (SEQ ID NO:6)

Paragraph spanning page 14, line 25 through page 15, line 2:

Plasmid pPS1134D4 was cleaved with *Bam*HI, and the following annealed oligonucleotides were cloned into that site:

PS1134C 5' GATCCGGCCTCCCGGGCC 3' (SEQ ID NO:7)
PS1134D 3' GCCGGAGGGCCCGGCTAG 5' (SEQ ID NO:8)

Paragraph on page 15 spanning lines 14 - 18:

DNA sequence derived from the nitroreductase gene (*nfnB*) of *E. coli* strain DH5 α was amplified by the polymerase chain reaction from genomic DNA purified from that strain, using primers PS1138A 5'- GGGAATTCCATATGGATATCATTTCTGTCGCCTTAAAGC-3' (SEQ ID NO:9) and PS1138B 5'- CGCGGATCCTGAGAGGAAATAGCCGGGCAGATGC -3' (SEQ ID NO:10).

Paragraph on page 17 spanning lines 17 - 24:

The polymerase chain reaction (PCR) was used to determine whether the bacteria in each colony contained λ JG3J1 or λ JG16C1. A number of individual colonies were picked at random from the most relevant plates and transferred to a 200 μ l PCR tube, lysed in a microwave for 2min and 35 μ l of PCR reaction mix [16mM (NH₄)₂SO₄, 67mM Tris-Cl (pH 8.8 at 25°C), 0.01% Tween-20 (Bioline), 0.2mM each dNTP, 1.5mM MgCl₂, 1[u] U Biotaq (Bioline)] plus 0.25 μ M primers JG2A 5'-TGGCGGAAAGGTATGCATGC-3' (SEQ ID NO:11) and; JG2B 5'-CAGAGCATTAGCGCAAGGTG-3' (SEQ ID NO:12), which anneal to λ sequences flanking the *Hind*III site, was added.

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3. **Murray, N. E.** (1983) [bacteriophage] Bacteriophage lambda and molecular cloning, p. 395-431. *In* Hendrix [(ed.)] et al., (eds.) Lambda II[], Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

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